Gel Electrophoresis of Restriction Digested LT10 Pro in PSB1C3 Plasmid

Made with Benchling

Project: Awesome Possum

Authors: Cara Jones

Date: 2016-09-26

MONDAY, 9/26

Purpose:

Separate digested LT10 Promoter constructs from PSB1C3 plasmid using gel electrophoresis.

Materials:

- 1.5 g of agarose powder
- 150.0 mL 1x TAE buffer
- Glass graduated cylinder
- Glass beaker
- Microwave
- Gel Red DNA stain
- Gel Electrophoresis apparatus
- 12 Well gel electrophoresis comb
- Digest DNA samples
- GeneRuler 1 Kbp plus ladder
- Transilluminator gel imager

Protocol:

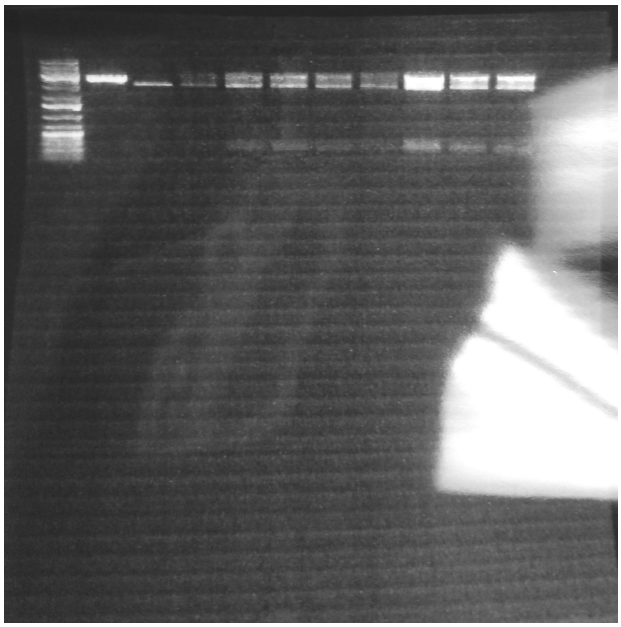
A 1.0% agarose gel was created by mixing 1.5 g agarose powder with 150.0 mL 1X TAE buffer in a glass beaker. Next, the mixture was microwaved until the solution was clear. After that, the beaker was placed on the lab bench and allowed to cool. Once the agarose mixture was cool enough, 15.0 µL of Gel Red stain was swirled into the contents. Then, a 12 well comb was inserted into an empty gel cassette mold and the gel mixture was poured into the electrophoresis apparatus. Once the gel reached an opaque appearance the comb was removed and the apparatus was filled with 1X TAE buffer until it flowed over the gel equally on both sides. After that, 10.0 µL of GeneRuler ladder was pipetted into Lane 1. After that, the samples (20.0 µL) were each pipetted into separate wells (see Table 1 for lane contents). Next, the gel was run at 100 volts for 90.0 minutes and then imaged using a transilluminator (see Figure 1).

Table 1: Gel Electrophoresis Lane Key

	A	B	C	D	E	F	G
1	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7
2	GeneRuler 1 Kbp Plus Ladder	RFP in PSB1C3 Single Digest	Colony 1 Single Digest	Colony 1 Double Digest	Colony 2 Double Digest	Colony 4 Double Digest	Colony 7 D Digest

Figure 1: Transilluminated Gel Electrophoresis Image

IMG1430335222 (1).jpg



1.0% Agarose Electrophoresis gel run for 90.0 minutes at 100 volts. Large light defect on right side of image from overhead light. Lane listing and contents provided in Table 1.

The gel was considered successful due to the existance of a second band in all of the double digest lanes. The second band represents the small LT10 Promoter construct fragment while the larger bands are cut PSB1C3 Vector. It is worth noting that the gel results should have been more spread out towards the bottom of the gel. The cause of the short length results was a faulty electrophoresis power supply. The power supply failed to keep a constant voltage throughout the run.